

PATENT COOPERATIVE TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 14 May 2001 (14.05.01)	
International application No. PCT/FI00/00733	Applicant's or agent's file reference 33781
International filing date (day/month/year) 30 August 2000 (30.08.00)	Priority date (day/month/year) 31 August 1999 (31.08.99)
Applicant SIPPONEN, Pentti et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 01 March 2001 (01.03.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Nestor Santesso Telephone No.: (41-22) 338.83.38
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REPLACED BY
ART 34 AMDT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 07 DEC 2001
WIPO PC

Applicant's or agent's file reference 33781	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/FI00/00733	International filing date (day/month/year) 30.08.2000	Priority date (day/month/year) 31.08.1999
International Patent Classification (IPC) or national classification and IPC ₇ C12Q 1/37, G01N 33/573		
Applicant Biohit OYJ et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 01.03.2001	Date of completion of this report 26.11.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Form PCT/IPEA/409 (cover sheet) (January 1998)	Authorized officer Fernando Farieta/BS Telephone No. 08-782 25 00

I. Basis of the report**1. With regard to the elements of the international application:***

- ☐ the international application as originally filed
- ☒ the description:
pages 1-9, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____
- ☒ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages 10, filed with the letter of 15.11.2001
- ☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____
- ☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims	<u>1-5</u>	YES
	Claims		NO
Inventive step (IS)	Claims	<u>1-5</u>	YES
	Claims		NO
Industrial applicability (IA)	Claims	<u>1-5</u>	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a method of screening by measuring from the serum both the pepsinogen I (SPGI) and the homocysteine concentration. A SPGI concentration below the method-specific cut-off value in combination above the reference value is an indication of an increased risk to fall ill with vascular and cancer disease.

Claim 1 has been supplemented with the letter of 2001-11-15, based on the description (page 4 lines 12-16).

During the search the following documents have been found relevant:

D1: Drugs & Aging, Vol 12, No 4 april 1998, Herman Nilsson-Ehle, "Age-related changes in Cobalamin (Vitamin B12) Handling".

D2: J. of Rheumatology, Vol 25 No 5 1998; Pettersson et al, "Serum homocysteine and methylmalonic acid in patients with rheumatoid arthritis and cobalaminopenia".

D3: Scand J Clin Lab Invest, Vol 54 No 219, 1994, Nexø et al, "How to diagnose cobalamin deficiency".

D4: WO 9615456 A1 (Locus Genex OY)

.../...

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

The determination of serum gastrin and pepsinogen levels reflects the great variation of health status in elderly individuals, according to D1. According to D2 there is a relationship between low pepsinogen A and atrophic gastritis in patients with suspected cobalamin deficiency. Documents D1 and D2 are referred to as representing the prior art.

Document D3 discloses vitamin B12 assays (e.g. Schilling test). The use of the test is to diagnose cobalamin deficiencies in patients with anemia. According to D3 results the sensitivity of a low P-pepsinogen A in a clinical material is related to the concentration cobalamin in serum for diagnose of gastric body atrophy (page 72).

A method for screening the risk of gastric cancer is presented in D4 (claims 1-12). The method comprises the determination of serum pepsinogen 1, gastrin-17 and antibodies in blood serum. The use of pepsinogen 1 concentration value in a method for identifying an individual at risk for cancer disease (in this case "gastric cancer") is known through document D4.

Claims 1-5

There is no indication in documents D3 or D4, that it would be possible to determine the risk for vascular and cancer disease based on the measured homocysteine and pepsinogen concentrations. According to D4 it is known that a low SPGI level is as such an independent risk factor for gastric cancer. The claimed invention is directed specifically to the combination of low SPGI and high homocysteine as being an indication of an increased oxygen stress on the body, a risk factor in connection with all types of cancer diseases.

To determine the concentration of pepsinogen 1 in order to use it as marker and the results with homocysteine measurements, using a cut-off value, which in combination is an indication of an increased risk to fall ill with vascular and cancer disease. It is not considered obvious to a person skilled in the art. Therefore, claims 1-5 are considered to fulfil the requirements of novelty and inventive step.

Claims

1. Method for identifying an individual at risk for vascular and cancer disease, the method comprising the steps of
 - 5 - determining quantitatively the pepsinogen I (PGI) analyte concentrations in a serum sample from said individual,
 - selecting a method specific cut-off value for the said analyte,
 - comparing the analyte concentration so determined to the method-specific cut-off value for the analyte, and
 - 10 - determining the homocysteine concentration in a serum sample from the individual, and comparing it to a method-specific reference value for homocysteine, whereby a serum pepsinogen I concentration below its method-specific cut-off value, and a serum homocysteine concentration above its method-specific reference value identifies the individual as an individual with an increased risk of
 - 15 vascular and cancer disease.
2. The method according to claim 1, comprising determining the serum PGI concentration, and selecting for further determination of the homocysteine concentration an individual who exhibits a serum PGI concentration below its cut-off value.
- 20 3. The method according to claim 1 or 2, comprising the further step of determining the B12 vitamin concentration in the sample and comparing it to a method-specific reference value.
- 25 4. Kit for carrying out the method according to claim 1, comprising
 - means for determining the PGI concentration in a serum sample,
 - means for determining the homocysteine concentration in a serum sample.
- 30 5. The kit according to claim 4 wherein the means for determining the pepsinogen I and homocysteine concentration comprise immunological means.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16356 A1

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- (21) International Application Number: PCT/FI00/00733
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- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- With international search report.
 - Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: METHOD FOR IDENTIFYING AN INDIVIDUAL AT RISK FOR VASCULAR AND CANCER DISEASE

(57) Abstract: The present invention is aimed at a method and a kit for identifying an individual at risk for vascular disease and cancer, the method comprising the steps of determining quantitatively the pepsinogen I (PGI) analyte concentrations in a serum sample from said individual, determining a method specific cut-off value for the said analyte, comparing the analyte concentration so determined to the method-specific cut-off value for the analyte, and determining the homocysteine concentration, in a serum sample from the individual.

WO 01/16356 A1

METHOD FOR IDENTIFYING AN INDIVIDUAL AT RISK FOR VASCULAR AND CANCER DISEASE

Field of the invention

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The present invention relates to a combination diagnostic method for identifying individuals who are at risk for coronary and vascular, as well as cancer diseases.

10

~~The method according to the invention is based on the combination of two tests~~
carried out on a blood or serum sample in order to identify individuals prone to develop or exhibiting elevated levels of homocysteine, which individuals are thus at risk of developing afflictions resulting from such elevated levels, such as cardio- and cerebrovascular disease, including atherosclerosis and ischaemic stroke, as well as cancer diseases.

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Background of the invention

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A number of different gastric diseases or conditions, such as chronic atrophic gastritis, pernicious anaemia, ventricular ulcer, gastric polyposis and the Ménétrier disease (giant hypertrophic gastritis) precede gastric cancer. Clearly identifiable changes of the mucosa are dysplasia and adenoma. It has been established that in almost all diseases the risk is mediated over chronic atrophic gastritis.

25

Chronic gastritis means a prolonged inflammatory condition of the gastric mucosa. The disease can coarsely be divided into the superficial and the atrophic form. In superficial gastritis, the inflammatory cell infiltration is concentrated below the surface epithelium. In case the inflammation progresses and diffuses between the specific gastric secretory glands, one refers to chronic atrophic gastritis. In such a case, the normal glandular structures of the gastric mucosa are at least partly substituted by metaplastic changes.

30

The relative risk of gastric cancer in patients suffering from atrophic gastritis in the corpus area of the stomach has been estimated, as calculated from the Finnish cancer statistics, to be about 4- to 5-fold as compared to persons having a healthy mucosa. In addition, there is a risk for falling ill with pernicious anaemia due to intrinsic factor deficiency and B12 vitamin absorption disturbance. In severe atrophy of the antrum area, the risk is even 18-fold. If atrophic changes appear both in the antrum and the corpus area (pangastritis), the risk can increase to even 90-fold.

10 The publication WO 96/15456 discloses a method for screening for the risk of gastric cancer according to which atrophy in the mucosa either of the corpus or the antrum area, or of both, is determined by determining the pepsinogen I (PGI) and gastrin-17 (G-17) analyte levels in a serum sample, and comparing the levels so determined to a method-specific cut-off value for respective analyte. The levels
15 determined are preferably also compared to a method-specific reference value for respective analyte.

A serum PGI value below the specific cut-off value for PGI indicates atrophic gastritis in the corpus area of the stomach. If the serum G-17 concentration is
20 below its cut-off value, the atrophy is located in the antrum area of the stomach. In pangastritis, the serum PGI is below the cut-off value and the serum G-17 value is at the lower limit of its reference value.

Methylenetetrahydrofolate reductase (MTHFR) is an intracellular enzyme that is
25 needed for remethylation of homocysteine to methionine. Impaired function of this enzyme is caused by defects in the structure of the MTHFR gene, or by nutritional deficiencies of *i.a.* folate, vitamin B6 and/or vitamin B12. Impaired function of the MTHFR enzyme results in an increase in the serum/plasma level of homocysteine (homocysteinemia) and in homocysteinuria. Increased serum/plasma
30 levels of homocysteine has in many studies shown to be associated with an increased risk for various coronary and vascular diseases, a high serum/plasma level

of homocysteine above the reference value being a serious independent risk factor for coronary and vascular disease and ischaemic stroke.¹⁻⁵

The atherogenic influence of homocysteine is thought to be based on an increased production of reactive oxygen species which enables lipid peroxidation.

The supply of sufficient B12 vitamin is necessary for folate metabolism and normal blood production, as well as for the function of the nerve cells. Vitamin B12 forms a complex with a protein, the intrinsic factor, produced by the mucosa of the corpus area of the stomach, which complex is resorbed in the lower part of the ileum. This is the preferred resorption form of vitamin B12.

A deficiency of intrinsic factor, as a result of atrophic gastritis or stomach cancer, especially in the corpus area of the stomach, will ultimately lead to a deficiency in B12 vitamin in the body and hence to an increase in the homocysteine concentration. It would thus be highly valuable to identify those subjects who have or are at a high risk for vitamin B12 deficiency due to atrophic gastritis, and who therefore might have an elevated serum or plasma level of homocysteine. In those persons an early initiation of vitamin B12 supplementation would be beneficial in the prevention of vascular diseases. It would also be valuable to be able to identify those individuals which would be at risk of cancer due to overproduction of intracellular oxygen radicals, and in whom a B12 vitamin supplementation or other treatment could be beneficial.

Summary of the invention

The present invention is directed to a method for combining an assay of determining, in a serum sample, a marker for atrophic gastritis in combination with an assay for homocysteine, in order to assist in the diagnosis of, or in the determination of the risk of vascular, as well as cancer diseases in an individual, the term vascular being understood broadly to include any coronary or vascular disease which can result from the atherogenic influence of homocysteine.

The method according to the invention is a method for identifying an individual at risk for vascular and cancer disease, the method comprising the steps of

- determining quantitatively the pepsinogen I (PGI) analyte concentrations in a serum sample from said individual,

5 - selecting a method specific cut-off value for the said analyte,
 - comparing the analyte concentration so determined to the method-specific cut-off value for the analyte, and

 - determining the homocysteine concentration in a serum sample from
10 the individual, and comparing it to a method-specific reference value for homocysteine.

The present invention thus allows for the identification of an individual having a serum pepsinogen I concentration below the method-specific cut-off value for serum pepsinogen I and a serum homocysteine concentration above the reference
15 value for homocysteine, as being an individual with an increased risk of, or having a predisposition for vascular and/or cancer disease.

The determination of the serum PGI and homocysteine concentrations can take place in any order, in order to simultaneously obtain knowledge of both the serum
20 PGI and homocysteine levels for the purpose of assisting in the diagnosis of, or assessing the risk of vascular disease or cancer. However, according to an embodiment of the invention, the serum PGI concentration is determined and compared to its determined or selected method-specific cut-off value, and for further diagnosis an individual is selected who has a serum PGI concentration value below its
25 method-specific cut-off value. In this embodiment, only the individuals which exhibit low serum PGI values indicative of corpus atrophy, are screened for homocysteine.

According to one embodiment of the invention, also the B12 concentration is
30 determined in the serum of the said individual, and compared to a method-specific reference value for vitamin B12.

The invention includes a step of comparing the measured analyte concentrations to method-specific cut-off or reference values for said analytes. The selection of such values is well known to a person skilled in the art, and depends on the specificity and sensitivity chosen for the test method used for the determination of the analyte concentrations, see e.g. William J Marshall, Clinical Chemistry, Third Edition, 1995, Mosby.

Thus according to a preferred embodiment, the present invention is especially aimed at screening such individuals, which still do not exhibit a B12 vitamin deficiency, that is which exhibit substantially normal B12 vitamin levels, but which due to low PGI values are diagnosed for atrophy in the corpus area of the stomach and which also have high homocysteine serum levels. Such an identification makes it possible to already at an early stage resort to preventative measures, for example to B12 supplementation, in order to counteract the development of the afflictions associated with elevated homocysteine levels.

Detailed description of the invention

1. Determination of pepsinogen I (PGI)

20

The method of determining PGI in a serum sample can be carried out as is described in the publication WO 96/15456, which publication is included herein for reference.

25

The said method preferably includes the steps of using poly- or monoclonal antibodies to pepsinogen I in an immunological method for the determination of pepsinogen I. Suitably the reaction is carried out on a suitable support, such as a plastic, glass or cellulose support, for example on a microplate. The immunological methods can be carried out in a known manner, using e.g. absorbance, luminescence or fluorescence techniques for measuring the said pepsinogen I concentration in the sample.

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If the serum pepsinogen I concentration is below the cut-off value, which depending on the specificity and sensitivity agreed upon for the method in question, is 20-30 $\mu\text{g/l}$, which corresponds to appr. 450 - 690 pmol/l , there is atrophy in the corpus area of the stomach. The normal or reference value for PGI is in the range of 25 - 120 $\mu\text{g/l}$.

2. Determination of homocysteine

Homocysteine levels in serum can be determined according to any of the methods known *per se* for this purpose and which are also commercially available, *e.g.* in kit form. The established method for quantifying total homocysteine in plasma or serum is high performance liquid chromatography with radioactive, fluorescent or electrochemical detection. Also an enzyme immunoassay (EIA) method has been developed (Bio-Rad Laboratories; Axis-Shield A/S) as well as a fluorescence polarisation immunoassay (FPIA; Abbott Laboratories), the immunoassay including pretreating the specimens with dithiothreitol and adenosine, followed by an enzymatic step to form S-adenosyl-L-homocysteine, and total homocysteine is measured *e.g.* using monoclonal anti-S-adenosyl-L-homocysteine antibodies, see *e.g.* US 5,631,127.

The reference values for homocysteine is to some degree method-specific, but generally varies between appr. 5 to 15 $\mu\text{mol/L}$. A serum homocysteine level which is above the method-specific reference level for homocysteine is taken as constituting a risk factor, as explained above. A homocysteine level of above 15 $\mu\text{mol/L}$ can in most cases be considered to constitute such an elevated level constituting a clear risk factor.

3. Determination of B12-vitamin

According to the invention, the method of diagnosis includes an optional method of determining the B12 vitamin concentration in the serum sample. The B12-vitamin (cobalamin) concentration can be determined according to any of the methods

per se known for this purpose. Such known methods include microbiological assay of serum B12 employing an organism, such as *Euglena gracilis* or *Lactobacillus leichmannii* which requires cobalamin for growth. Also radioisotope dilution assays for B12 vitamin have been utilized and such assay techniques are well documented in the literature, e.g. Lau *et al.*, "Measurement of serum B12 levels using Radioisotope Dilution and Coated Charcoal", Blood, 26 (1965), 202. Radioisotope dilution methods are more rapid and give results comparable with those of e.g. the *Euglena* assay, provided the binding protein is specific for biologically active cobalamin. A standardized pure or purified intrinsic factor preparation is most satisfactory as the binding protein as it binds specifically to true cobalamin rather than cobalamin analogues.

The radioisotope dilution assay of B12 generally includes the step of freeing endogenous B12 from its natural binding protein e.g by boiling at a selected pH and then adding a measured amount of the radioisotope ⁵⁷Co-B12, and a limited amount of binding protein. All of the binding protein will be bound by some form of B12 since the amount of radioisotope B12 added is sufficient to bind the small amount of protein. As both the natural and the radioactive B12 compete to bind with the binding protein, the degree to which the radioactive count of the protein bound B12 was inhibited is indicative of the amount of B12 in the sample. This method has been modified by Lau, *supra*, by separating unbound B12 from protein bound B12 by protein coated charcoal and the radioactivity of the supernatant liquid containing the mixture of bound radioactive B12 and bound non-radioactive B12 is counted for radioactivity. The serum B12 concentration is then calculated from the count, often by comparison with a standard chart. Radioassay kits are commercially available for carrying out the method.

B12 vitamin deficiency has also been determined using e.g. chemiluminescence receptor assays (Wentworth, S. *et al.*, Clin. Chem., vol 40 537-540), radioimmunoassays (Endres, D.B., *et al.* Clin. Chem., Vol. 24, 460-465) as well as nonisotopic binding assays, CEDIA, cloned enzyme donor immunoassays (van der Weide, J. *et al.* Clin. Chem., Vol. 38, 766-768).

The reference value for B12 varies between 200-900 ng/l, corresponding to appr. 170 to 700 pmol/l.

5 In our recent unpublished work we found that 50% of those subjects suffering from corpus atrophy (SPGI < 25 μ g/l) whose vitamin B12 serum concentration was below the lower part of the reference limit (< 170 pmol/l) had markedly increased serum homocysteine concentration (mean 33.3 μ mol/l, 16-157 μ mol/l).

10 In addition, 22% of those subjects whose vitamin B12 serum concentration was between 180 - 230 pmol/l (reference values 170 - 700 pmol/l) had also an increased homocysteine concentration (mean 18.9 μ mol/l, 16 - 25 μ mol/l).

The present invention also relates to a kit for use in the method according to the invention the kit comprising

- 15 - means for determining the PGI concentration in a serum sample,
- means for determining the concentration of homocysteine in a serum sample.

20 The kit according to the invention can comprise a combination of the individual components needed to quantitatively determine the serum pepsinogen I and the homocysteine concentration in a blood serum sample. For this purpose the kit can comprise separate vials or containers for the necessary components, such as antibodies and substrates to be used for the determination of the analyte.

References

1. Boushey, C.J., Beresford, S.A.A., Omenn, G.S., Motulsky, A.G., JAMA 1995; 274:1049-57
2. Graham, I.M., Daly, L.E., Rafsum, H.M. *et. al.*, The European Concerted Action Project, JAMA 1997; 277:1775-81
3. Jacobsen, O.W., Clin Chem 1998; 44:1833-43
4. Mogadashian, M. H., McManus, B.M., Frohlich, J.J., Arch Intern Med 1997; 157: 2299-2308
5. Rafsum, H., Ueland, P.M., Nygård, O., Vollset, S.E., Rev Medicine 1998; 49:31-62.

Claims

1. Method for identifying an individual at risk for vascular and cancer disease, the method comprising the steps of
- 5 - determining quantitatively the pepsinogen I (PGI) analyte concentrations in a serum sample from said individual,
- selecting a method specific cut-off value for the said analyte,
- ~~comparing the analyte concentration so determined to the method-~~
specific cut-off value for the analyte, and
- 10 - determining the homocysteine concentration in a serum sample from the individual, and comparing it to a method-specific reference value for homocysteine.
2. The method according to claim 1, comprising determining the serum PGI concentration, and selecting for further determination of the homocysteine concentration an individual who exhibits a serum PGI concentration below its cut-off value.
- 15
3. The method according to claim 1 or 2, comprising the further step of determining the B12 vitamin concentration in the sample and comparing it to a method-
- 20 specific reference value.
4. Kit for carrying out the method according to claim 1, comprising
- means for determining the PGI concentration in a serum sample,
- means for determining the homocysteine concentration in a serum sample.
- 25
5. The kit according to claim 4 wherein the means for determining the pepsinogen I and homocysteine concentration comprise immunological means.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00733

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/37, G01N 33/573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Drugs & Aging, Volume 12, No 4, April 1998, Herman Nilsson-Ehle, "Age-Related Changes in Cobalamin (Vitamin B12) Handling. Implications for Therapy", page 277 - page 292, figure 1 --	1-5
X	J. of rheumatology, Volume 25, No 5, 1998, Tom Pettersson et al, "Serum Homocysteine and Methylmalonic Acid in Patients with Rheumatoid Arthritis and Cobalaminopenia" page 859 - page 863 --	1-5
A	Scand J Clin Lab Invest, Volume 54, No 219, 1994, Ebba Nexø et al, "How to diagnose cobalamin deficiency", page 61 - page 76, pages 10, 12 --	1-5

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00733

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 9615456 A1 (LOCUS GENEX OY), 23 May 1996 (23.05.96), pages 1-4</p> <p style="text-align: center;">-- -----</p>	1-5

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Information on patent family members

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